Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Fish (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 47807002

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	367525
OECD Data Point	OECD Guideline 203
EPA MRID	47807002
EPA Guideline	OPPTS 850.1075

Test material: Trifluralin Metabolite TR-15 **Purity:** 99%
Common name: Trifluralin
Chemical name: IUPAC:1h-benzimidazole,2-ethyl-4-nitro-6-(trifluoromethyl))
CAS name: Not Reported
CAS No.: Not Reported
Synonyms: None Reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 11/09/09

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 12/01/09

Primary Reviewer: **Christine Hartless** **Date:** 02/25/10
{EPA/OPP/EFED/ERB1} 2-25-10

Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA/OECD/PMRA}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	036101	

Date Evaluation Completed: {02-25-10}

CITATION: Marino, T.A., C.A. Hales, E.L. McClymont, and A.M. Yaroch. 2001. Trifluralin Metabolite TR-15: An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory report number 011106. Study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana. Study completed September 11, 2001.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss* Walbaum) were exposed to trifluralin metabolite TR-15 at nominal concentrations of 0 (negative and solvent controls), 1.01, 1.68, 2.81, 4.68, 7.80, and 13.0 mg ai/L under static conditions. The 96-hour mean-measured concentrations were <0.09 (LOQ; controls), 1.04, 1.73, 2.84, 4.69, 7.77, and 13.0 mg ai/L. The 96-h LC_{50} was 6.04 mg ai/L. The NOAEC value, based on mortality and sub-lethal effects, was 1.04 mg ai/L. With the exception of the lowest treatment level (1.04 mg ai/L), sub-lethal effects (e.g., partial or complete loss of equilibrium, lethargy, erratic swimming, ascites and immobility) were observed in all groups exposed to the trifluralin metabolite TR-15. Based on the results of this study, trifluralin metabolite TR-15 would be classified as moderately toxic to rainbow trout in accordance with the classification system of the U.S. EPA.

This toxicity study is scientifically sound and classified as ACCEPTABLE (for the degradate TR-15) based on the guideline requirements for an acute freshwater fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean weight or length): juveniles, mean length and weight of surviving fish at test termination were 45 mm and 858 mg, respectively.
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: 6.04 mg ai/L 95% C.I.: 1.73-7.77 mg ai/L (binomial method)
Probit Slope: N/A 95% C.I.: N/A

NOAEC: 4.69 mg ai/L (statistically determined based on mortality)
NOAEC: 1.04 mg ai/L (visually determined based on sub-lethal effects and mortality)

Endpoint(s) Affected: mortality and sub-lethal effects

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals Number 203, "Fish, Acute Toxicity Test", and the European Economic Community (EEC) Method C.1, Acute Toxicity for Fish. Methods were also in general accordance with procedures put forth by the U.S. EPA. The following deviations from OPPTS 850.1075 were noted:

1. Aluminum, iron, and zinc were detected in the dilution water at concentrations of 0.038, 0.069, and 0.037 mg/L, which exceeded the maximum allowable concentration of 0.001 mg/L.
2. The measured hardness of the dilution water (36 mg/L as $CaCO_3$) fell below the recommended range (40-180 mg/L as $CaCO_3$).

These deviations do not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with the following GLP Standards: OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98(17; EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999); and Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Good Laboratory Practice Standards, Final Rule.

A. MATERIALS:

1. Test material Trifluralin Metabolite TR-15

Description: Solid

Lot No./Batch No. : GHD-6140-43C

Purity: 99%

Stability of compound under test conditions: Analytical verification of samples collected at test initiation yielded recoveries of 100.8 to 105% of nominal. Samples collected at test termination yielded recoveries of 98.3 to 103.6% of nominal and 96.8 to 101.2% of initial measured concentrations. The resulting 96-hr mean-measured concentrations resulted in recoveries of 99.6 to 103% of nominal, indicating that the test material was stable during the definitive exposure period.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not Reported

Physicochemical properties of Trifluralin Metabolite TR-15.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss* Walbaum) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: Juveniles

Weight at study initiation: 858 mg, based on surviving fish at test termination
EPA recommends: mean 0.5 - 5 g.

Length at study initiation: 45 mm, based on surviving fish at test termination *EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∀ 1.0 cm for bluegill and 5.0 ∀ 1.0 cm for rainbow trout*

Source: Thomas Fish Company, Anderson, California
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 96-hour range-finding study was conducted using nominal concentrations of 0 (negative control), 0.190, 1.90, 9.50, 19.0, and 100 mg ai/L. The LC_{50} value was determined to be between 1.90 and 9.50 mg ai/L. These results were used to determine the nominal concentrations for the definitive exposure test.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	At least 14 days Same as test Fish received a standard diet (Aquatic Diet Number 1, Lot #992236, Harlan-Teklad, Madison, Wisconsin) at least once daily. Feeding was terminated 48 hours prior to test initiation. Mortality was <5% during the 48 hours prior to test initiation.	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Duration of the test	96 hours	*The recommended test duration is 96 hours.*
Test condition Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	Static N/A N/A	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	Aeration was provided at approximately 100 bubbles/minute.	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass beakers 12 L 10 L	*Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm. Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Laboratory water is Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant. The water is limed and flocculated with ferric chlorides. Prior to use, the water is filtered, UV-irradiated, and pH-adjusted with CO_2.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	36 mg/L as $CaCO_3$ 7.0-7.7 8.8-10.6 mg/L (≥85% saturation) <1000 ng/mL <1000 ng/mL See Reviewer's Comments None Detected <100 ng/mL (residual chlorine) 12.8-13.5°C N/A Temperature, DO, and pH were measured at test initiation and every 24 hours thereafter in every test vessel containing surviving fish. Temperature was also continuously monitored in one test vessel throughout the study.	*Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ∃60% during first 48 hrs and ∃40% during second 48 hrs; flow-through: ∃60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 1 1 1/level	*Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 10 10	*Number of organisms per replicate should be ∃10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.429 g/L	*Recommended static conditions are # 0.8 g/L at # 17EC and # 0.5 g/L at > 17EC. Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: nominal: measured:	0 (negative and solvent controls), 1.01, 1.68, 2.81, 4.68, 7.80, and 13.0 mg ai/L <0.09 (LOQ; controls), 1.04, 1.73, 2.84, 4.69, 7.77, and 13.0 mg ai/L,	
Solvent (type, percentage, if used)	DMF (0.1 mL/L)	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D with a transition period of low light intensity	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the definitive exposure.	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination Level of quantization Level of detection	 0 and 96 hours 0.09 mg ai/L Not Reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not included in the study	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	-mortality -sub-lethal effects	
Observation intervals	24, 48, 72, and 96 hours	
		Observation intervals should be a minimum of every 24 hours.
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Mortality was first observed after 24 hours in the mean-measured 2.84, 7.77, and 13.0 mg ai/L treatment groups; mortality was 10, 20, and 100%, respectively, yielding a 24-hour LC_{50} value of 8.63 (7.34-10.2) mg ai/L. After 48 hours of exposure, mortality was 10, 50, and 100% in the mean-measured 2.84, 7.77, and 13.0 mg ai/L treatment groups, respectively, yielding a 48-hr LC_{50} value of 7.22 (5.69-9.39) mg ai/L. After 72 hours, mortality was 10, 90, and 100% in the mean-measured 2.84, 7.77, and 13.0 mg ai/L treatment groups, respectively, yielding a 72-hr LC_{50} value of 5.88 (4.72-7.34) mg ai/L. By test termination, mortality was 0% in the controls and mean-measured 1.04, 1.73, and 4.69 mg ai/L treatment groups, and 20, 100, and 100% in the mean-measured 2.84, 7.77, and 13.0 mg ai/L treatment groups, respectively, yielding a 96-hr LC_{50} value of 5.46 (4.77-6.25) mg ai/L. The study authors determined the 96-hr NOAEC value to be 1.04 mg ai/L, the highest treatment level with no mortality and/or sub-lethal effects.

Table 3: Effect of Trifluralin Metabolite TR-15 on Mortality of *Oncorhynchus mykiss*.

Mean-Measured and (Nominal) Concentrations mg ai/L	No. of Fish at Start of Study	Observation Period					
		Day 1		Day 3		Day 4	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	10	0	0	0	0	0	0
Solvent Control	10	0	0	0	0	0	0
1.04 (1.01)	10	0	0	0	0	0	0
1.73 (1.68)	10	0	0	0	0	0	0
2.84 (2.81)	10	1	10	1	10	2	20
4.69 (4.68)	10	0	0	0	0	0	0
7.77 (7.80)	10	2	20	9	90	10	100
13.0 (13.0)	10	10	100	10	100	10	100
NOAEC	1.04 mg ai/L						
LC_{50}	24-hrs: 8.63 (7.34-10.2) mg ai/L 48-hrs: 7.22 (5.69-9.39) mg ai/L 72-hrs: 5.88 (4.72-7.34) mg ai/L 96-hrs: 5.46 (4.77-6.25) mg ai/L						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. NON-LETHAL TOXICITY ENDPOINTS:

Throughout the definitive exposure period, no sub-lethal effects were observed in the controls or mean-measured 1.04 mg ai/L treatment group. Effects observed in the remaining treatment groups included partial loss of equilibrium, complete loss of equilibrium, immobility, lethargy ascites, and erratic swimming.

After 24 hours of exposure, 10% of the surviving fish at the 1.73 mg ai/L treatment level exhibited a partial loss of equilibrium. At the 2.84 mg ai/L treatment level, one and two of the nine surviving fish exhibited lethargy and a partial loss of equilibrium, respectively. At the 4.69 mg ai/L treatment level, lethargy, erratic swimming, and a partial loss of equilibrium were each observed in a different fish. At the 7.77 mg ai/L treatment level, six of the eight surviving fish were exhibiting effects, which included complete loss of equilibrium ascites, partial loss of equilibrium, and lethargy. All fish at the 13.0 mg ai/L treatment level were dead by 24 hours.

After 48 hours, lethargy and a partial loss of equilibrium were observed in two and one of the nine surviving fish, respectively, at the 2.84 mg ai/L treatment level. At the 4.69 mg ai/L treatment level, 20 and 40% of the surviving fish were observed with lethargy and a partial loss of equilibrium, respectively. At the 7.77 mg ai/L treatment level, 60% of the surviving fish were lethargic and 20% exhibited ascites and a complete loss of equilibrium.

After 72 hours, one fish (10%) at the mean-measured 1.73 mg ai/L treatment level exhibited a partial loss of equilibrium. At the 2.84 mg ai/L treatment level, one fish was lethargic, one exhibited a partial loss of equilibrium, and one was both lethargic and exhibiting a partial loss of equilibrium. At the 4.69 mg ai/L treatment level, five fish were experiencing a partial loss of equilibrium and two were lethargic with a partial loss of equilibrium. At the 7.77 mg ai/L treatment level, the one surviving fish exhibited a partial loss of equilibrium.

By test termination, one fish (10%) at the 1.73 mg ai/L treatment level was observed with a partial loss of equilibrium. At the 4.69 mg ai/L treatment level, two fish exhibited a partial loss of equilibrium and two other fish exhibited a partial loss of equilibrium and lethargy. All fish at the two highest treatment levels were dead.

Table 4: Sub-lethal Effects of Trifluralin Metabolite TR-6 on *Oncorhynchus mykiss*.

Mean-Measured and (Nominal) Concentrations mg ai/L	Observation Period		
	Effects at 24 Hrs	Effects at 72 Hrs	Effects at 96 Hrs
	% Affected	% Affected	% Affected
Negative Control	A.N.	A.N.	A.N.
Solvent Control	A.N.	A.N.	A.N.
1.04 (1.01)	A.N.	A.N.	A.N.
1.73 (1.68)	10%- PE	10%- PE	10%- PE
2.84 (2.81)	11%- L 22%- PE	11%- L 22%- PE 11%- PE, L	25%- PE 25%- PE, L
4.69 (4.68)	10%- L 10%- ES 10%- PE	50%- PE 20%- PE, L	60%- PE 20% PE, L
7.77 (7.80)	38%- L 13%- AS 13%- PE 13%- CE, I, AS	100%- PE	--
13.0 (13.0)	--	--	--
NOAEC	1.04 mg ai/L	1.04 mg ai/L	1.04 mg ai/L
LOAEC	1.73 mg ai/L	1.73 mg ai/L	1.73 mg ai/L
EC_{50}	Not Reported	Not Reported	Not Reported
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A

A.N.- all surviving fish appeared normal and healthy
PE- partial loss of equilibrium
CE- complete loss of equilibrium

L- lethargy
AS- ascites
I- immobile
ES- erratic swimming
N/A- not applicable

C. REPORTED STATISTICS:

The U.S. EPA Probit Program, Version 1.5, using mean-measured concentrations was use to calculate the LC_{50} values, confidence intervals, and probit slopes. If the Probit Program could not be used, then the U.S. EPA Trimmed Spearman-Karber (TSK) Program, Version 1.5 was used to calculate the LC_{50} values and the corresponding trim values. The NOAEC value was visually determined based on the highest exposure level that exhibited 0% mortality or sub-lethal effects.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Cumulative mortality data were analyzed using the binomial probability method via Toxanal statistical software to determine the 96-hr LC_{50} value and the associated 95% confidence limits. The NOAEC value based on mortality was determined using Fisher's Exact Test via Toxstat statistical software. The overall NOAEC was determined by considering the results of the Fisher's test, as well as the dose-responses of both mortality and the occurrence of sub-lethal effects. All toxicity values were determined using the 96-hr mean-measured concentrations.

LC_{50}: 6.04 mg ai/L 95% C.I.: 1.73-7.77 mg ai/L (binomial method)
Probit Slope: N/A 95% C.I.: N/A

NOAEC: 4.69 mg ai/L (statistically determined based on mortality)
NOAEC: 1.04 mg ai/L (visually determined based on sub-lethal effects and mortality)

E. STUDY DEFICIENCIES:

No deficiencies were noted.

F. REVIEWER'S COMMENTS:

The study author's and reviewer's estimates of the LC_{50} were very similar. The reviewer's toxicity values are reported in the Executive Summary and Conclusions sections of this DER.

The results from the most recent periodic screening analysis of the laboratory dilution water indicated the presence of the following inorganics: aluminum (38 ng/mL), calcium (17,000 ng/mL), iron (69 ng/mL), magnesium (8,600 ng/mL), potassium (1,100 ng/mL), sodium (4,800±200 ng/mL), zinc (37 ng/mL), bromide (30±1 ng/mL), fluoride (110 ng/mL), nitrate (1,100 ng/mL), phosphate (80 ng/mL), and sulfate (17,000 ng/mL).

The reviewer's statistical analysis of the mortality data yielded a NOAEC value of 4.69 mg ai/L. However, the reviewer felt that the 20% mortality at the next lowest treatment level was biologically significant, and taking into consideration the sub-lethal effects, visually determined the overall NOAEC value to be 1.04 mg ai/L.

The in-life portion of the definitive toxicity test was conducted from June 18 to June 22, 2001.

G. CONCLUSIONS:

This toxicity study is scientifically sound and classified as ACCEPTABLE (for the degradate TR-15) based on the guideline requirements for an acute freshwater fish toxicity study.
The NOAEC and LC_{50} values were determined to be 1.04 and 6.04 mg ai/L, respectively. Based on the results of this study, trifluralin metabolite TR-15 would be classified as moderately toxic to rainbow trout in accordance with the classification system of the U.S. EPA.

LC_{50}: 6.04 mg ai/L 95% C.I.: 1.73-7.77 mg ai/L (binomial method)
Probit Slope: N/A 95% C.I.: N/A

NOAEC: 4.69 mg ai/L (statistically determined based on mortality)
NOAEC: 1.04 mg ai/L (visually determined based on sub-lethal effects and mortality)

Endpoint(s) Affected: mortality and sub-lethal effects

III. REFERENCES:

Organization for Economic Cooperation and Development (OECD). OECD Guidelines for Testing of Chemicals, Method 203, "Fish, Acute Toxicity Test", ISBN 92-64-12221-4. Adopted July, 1992.

Official Journal of the European Communities. European Economic Community (EEC) Method C.1. Acute Toxicity for Fish. ISSN 0378-6978. December 1992.

EPA-FIFRA. Environmental Protection Agency. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test for Fish. EPA-540/9-85-006. June 1985.

U.S. Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-1, Acute Toxicity Test for Freshwater Fish. EPA-540/09-87-198. December 1986.

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

EC Directive 99/11/EC of 8 March (OJ No. L 77/8-21, 23/3/1999).

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160- Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

Dow AgroSciences LLC Test Substance Distribution Certificat. TSN102443, Dow AgroSciences LLC, Indianapolis, Indiana, 30 March 2001.

Madsen, S. Certificate of Analysis for Test/Reference/Control/Substances, FA&PC Number 013014, Dow AgroSciences LLC, Indianapolis, Indiana, 19 March 2001.

McClymont, E.L. and M.S. Mielke. Analytical Data for Trifluralin Metabolite TR-6: Growth Inhibition Test with the Fresh Water Green Alga, *Selenastrum capricornitum*, Printz, Study #011101, Report in Progress.

Probit Program Version 1.5, U.S. EPA, 1994.

Trimmed Spearman-KArber (TSK) Program, Version 1.5, U.S. EPA, 1994.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
13	10	10	100	9.765625E-02
7.77	10	10	100	9.765625E-02
4.69	10	0	0	9.765625E-02
2.84	10	2	20	5.46875
1.73	10	0	0	9.765625E-02
1.04	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 1.73 AND 7.77 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 6.036663

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	1.04	10	0	
2	1.73	10	0	
3	2.84	10	2	
4	4.69	10	0	
5	7.77	10	10	*
6	13	10	10	*